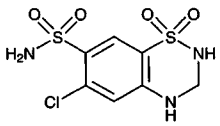
REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Hydrochlorothiazide



Molecular formula: $C_7H_8ClN_3O_4S_2$
Molecular weight: 297.74
CAS Registry No.: 58-93-5
Merck Index: 4822
Lednicer No.: 1 358

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum +100 µL 1.25 µg/mL IS + 5 mL MTBE, vortex for 2 min. Centrifuge at 2700 g for 5 min and evaporate the organic phase to dryness under a stream of nitrogen. Dissolve the residue in 200 µL water, add 3 mL toluene, vortex for 2 min, centrifuge at 2700 g for 10 min, discard the toluene layer . Add 3 mL toluene, vortex, centrifuge, discard the toluene layer. Evaporate the aqueous layer to dryness under a stream of nitrogen. Reconstitute the residue in 200 µL mobile phase. Inject a 100 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm RP-C18
Column: 250 × 4 5 µm LiChrospher 100 RP-C18
Mobile phase: MeCN:THF:200mM pH 7.5 phosphate buffer 5:10:85
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 273

CHROMATOGRAM
Internal standard: hydroflumethiazide
Limit of detection: 3.3 ng/mL
Limit of quantitation: 11.2 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Vervaet,C.; Remon,J.P. Bioavailability of hydrochlorothiazide from pellets, made by extrusion/spheronisation, containing polyethylene glycol 400 as a dissolution enhancer, *Pharm.Res.*, **1997**, *14*, 1644–1646.

SAMPLE
Matrix: blood
Sample preparation: 200 µL Serum + 20 µL 1 M pH 7.0 phosphate buffer, extract with 5 mL MTBE, vortex for 20 s, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 10 µL 20 µg/mL IS in MeOH, evaporate to dryness at 80° in a vacuum centrifuge, reconstitute the residue with 180 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 µm endcapped LichroCART RP18
Mobile phase: MeCN:7.5 mM pH 7.3 phosphate buffer 10:90
Column temperature: 40
Flow rate: 0.8
Injection volume: 30
Detector: E, ESA Coulochem II, coulometric cell 5011, first electrode +450 mV, second electrode +630 mV; UV 254

CHROMATOGRAM
Retention time: 5.8
Internal standard: p-aminobenzoic acid (1.2)
Limit of quantitation: 5 ng/mL (E)

KEY WORDS
serum

REFERENCE
Richter,K.; Oertel,R.; Kirch,W. New sensitive method for the determination of hydrochlorothiazide in human serum by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 293–296.

SAMPLE
Matrix: blood, formulations
Sample preparation: Serum. Condition a Bakerbond C18 SPE cartridge with 3 mL MeOH and 3 mL water. Mix 100 μL serum with 200 μl MeCN, vortex for 2 min, add 100 μL 4.08 μg/mL IS in MeOH, mix, centrifuge at 4000 rpm for 15 min. After the removal of the organic solvent add the supernatant to the SPE cartridge, dry under vacuum, wash with 3 mL water, elute with 3 mL MeOH. Evaporate to dryness under a stream of nitrogen at 45°, dilute to 100 μL with MeOH. Inject a 20 μL aliquot. Tablets. Powder tablets. Prepare an 1-3 μg/mL solution of hydrochlorothiazide in MeOH containing 4.08 μg/mL IS. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:1% acetic acid 20:80
Flow rate: 1.3
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 3.146
Internal standard: hydroflumethiazide (5.28)
Limit of detection: 50 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: captopril

KEY WORDS
tablets; plasma; SPE

REFERENCE
Papadoyannis,I.N.; Samanidou,V.F.; Georga,K.A.; Georgarakis,E. High performance liquid chromatographic determination of hydrochlorothiazide (HCT) in pharmaceutical preparations and human serum after solid phase extraction, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1671–1683.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 226.3

CHROMATOGRAM
Retention time: 9.397

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Dilute 25 mL 9.6 mg/mL chlorothiazide in MeOH with 1 mL 380 mg/L IS in 0.1% phosphoric acid, inject an aliquot.

HPLC VARIABLES
Column: A 250 × 2 J sphere ODS-M80; B 150 × 4.6 5 μm Beckman Ultrasphere C18
Mobile phase: A Gradient. MeCN:0.1% formic acid from 0:100 to 30:70 over 20 min. B Gradient. MeCN:0.1% phosphoric acid 0:100 to 30:70 over 12 min.
Flow rate: A 0.2; B 1
Detector: A MS, Finnigan Model TSQ-7000 triple-quadrupole, nebulizer nitrogen 260°; B UV 270

CHROMATOGRAM
Retention time: 22
Internal standard: ethylparaben

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
photolysis

REFERENCE
Revelle,L.K.; Musser,S.M.; Rowe,B.J.; Feldman,I.C. Identification of chlorothiazide and hydrochlorothiazide UV-A photolytic decomposition products, *J.Pharm.Sci.*, **1997**, *86*, 631–634.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS1
Mobile phase: MeOH:50 mM pH 3.0 phosphoric acid 10:90
Column temperature: 30
Flow rate: 1.5
Detector: radioactivity detection

OTHER SUBSTANCES
Also analyzed: atenolol, cimetidine, ranitidine

KEY WORDS
14C labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

SAMPLE
Matrix: urine
Sample preparation: Dilute 10 mL urine to 15 mL with water, add to Extrelut-20 cartridge, elute with 60 mL ethyl acetate:isopropanol 85:15. Evaporate under vacuum at 50°, filter, dry under nitrogen, reconstitute the residue in 100 µL acetone. Add 100 µL 1 mg/mL 3-bromomethylpropylphenazone in acetone, mix with 1 mg anhydrous potassium carbonate, make up to 200 µL with acetone. Let stand at 105 ± 5° for 60 min. Cool the reaction mixture, dry under a gentle stream of nitrogen. Reconstitute the residue with 500 µL MeCN, shake for 2 min. inject a 10 µL aliquot. (3-Bromomethylpropylphenazone is produced by the reaction of propylphenazone with bromine and recrystallized from chloroform:diethyl ether 1:2. (Caution! Chloroform is a carcinogen!))

HPLC VARIABLES
Column: 250 × 4.6 6 µm Zorbax C8
Mobile phase: MeCN:MeOH:50 mM sodium acetate 34:8:28, adjusted to pH 6.5 with acetic acid
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 254

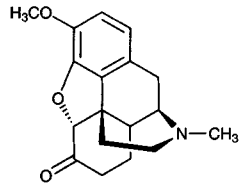
CHROMATOGRAM
Retention time: 27.7 (derivatized), 3.3 (underivatized)

OTHER SUBSTANCES
Extracted: captopril

KEY WORDS
derivatization; SPE

REFERENCE
Khedr,A.; El-Sherief,H. 3-Bromomethyl-propyphenazone as a new derivatization reagent for high performance liquid chromatography of captopril and hydrochlorothiazide with UV-detection, *Biomed.Chromatogr.*, **1998**, *12*, 57–60.

Hydrocodone



Molecular formula: $C_{18}H_{21}NO_3$
Molecular weight: 299.37
CAS Registry No.: 125-29-1, 34195-34-1 (bitartrate hydrate), 143-71-5 (bitartrate)
Merck Index: 4826
Lednicer No.: 1 288

SAMPLE
Matrix: formulations
Sample preparation: 500 µL or 1.0 mL Sample + 1.0 mL water + 500 µL 1 M NaOH + 15 mL dichloromethane, shake at 100 cpm for 20 min. Centrifuge at 2500 rpm for 5 min, evaporate organic layer to dryness under a gentle stream of nitrogen at 35 to 40°. Dissolve residue in 5 mL MeOH, inject a 20 to 80 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 µm Alltech C8

Mobile phase: MeCN:pH 4.5 buffer 18:82 (Buffer was 10 mM KH_2PO_4 and 50 mM potassium nitrate.)
Flow rate: 1.4
Injection volume: 20-80
Detector: UV 280

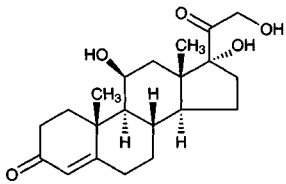
CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Noninterfering: chorpheniramine

KEY WORDS
suspensions

REFERENCE
Hadzija,B.W.; Shrewsbury,R.P. Determination of hydrocodone in Tussionex extended-release suspension by high-performance liquid chromatography (HPLC), *J.Forensic Sci.*, **1996**, *41*, 878–880.

Hydrocortisone



Molecular formula: $C_{21}H_{30}O_5$
Molecular weight: 362.47
CAS Registry No.: 50-23-7, 13609-67-1 (butyrate), 57524-89-7 (valerate), 50-03-3 (acetate), 3863-59-0 (phosphate), 6000-74-4 (sodium phosphate), 125-04-2 (21-sodium succinate), 508-96-3 (tebutate), 74050-20-7 (aceponate), 72590-77-3 (buteprate), 508-99-6 (cypionate), 83784-20-7 (hemisuccinate monohydrate), 2203-97-6 (hemisuccinate), 2203-97-6 (succinate), 5752489-7 (valerate)
Merck Index: 4828
Lednicer No.: 1 190

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge. Mix 1 mL plasma with 134.0 ng hydrocortisone-d_5 and 74.56 ng cortisone-d_5. Add the sample to the SPE cartridge, wash with 8 mL water, elute with 4 mL ethyl acetate, evaporate the eluate to dryness at 70° under a stream of nitrogen, dissolve the residue in 30 μL mobile phase, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.0 4 μm LiChroCART Superspher 100
Mobile phase: A MeOH:THF:50 mM ammonium formate 17:53:180; B MeCN:50 mM ammonium formate 35:65
Flow rate: 0.6 (A); 1.3 (B)
Injection volume: 20
Detector: MS, Shimadzu LCMS-QP1000EX Model 750 B, thermospray, vaporizer control 155°, vaporizer tip 195°, vapor 274°, ion source block 295°, tip heater 305°, m/z 363

CHROMATOGRAM
Retention time: 13 (A)
Internal standard: hydrocortisone-d_5, cortisone-d_5
Limit of detection: 0.25 ng

OTHER SUBSTANCES
Extracted: cortisone, prednisolone, prednisone

KEY WORDS
plasma; SPE

REFERENCE

Shibasaki,H.; Furuta,T.; Kasuya,Y. Quantification of corticosteroids in human plasma by liquid chromatography-thermospray mass spectrometry using stable isotope dilution, *J.Chromatogr.B*, **1997**, *692*, 7–14.

SAMPLE

Matrix: blood

Sample preparation: Add 100 µL MeOH and 50 µL 1 µg/mL fluocortolone in MeOH to 1 mL plasma. Add 500 µL 100 mM NaOH and 2 mL dichloromethane, shake for 10 min, centrifuge at 2500 g for 10 min, evaporate a 1.9 mL aliquot of the supernatant under a stream of nitrogen at 45°. Reconstitute the residue in 50 µL MeOH, inject 17 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 5 µm LiChrospher RP 18
Column: 250 × 4 5 µm Lichrospher RP 18
Mobile phase: MeOH:THF:water 110:2.5:100
Flow rate: 1
Injection volume: 17
Detector: UV 252

CHROMATOGRAM

Internal standard: fluocortolone
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: triamcinolone

KEY WORDS

plasma

REFERENCE

Doppenschmitt,S.A.; Scheidel,B.; Harrison,F.; Surmann,J.P. Simultaneous determination of triamcinolone acetonide and hydrocortisone in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *682*, 79–88.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Serum or urine + 500 µL 200 mM pH 3.85 acetate buffer (serum only) + 400 µL 2.5 µM IS in mobile phase, mix, centrifuge. Add the supernatant to the SPE cartridge, wash with 3 mL acetone:water 20:80, 3 mL water, and 3 mL hexane. Elute with 3 mL diethyl ether into tubes containing 1 mL 200 mM NaOH, vortex, centrifuge. Dry the organic layer under a stream of nitrogen. Reconstitute the residue in 250 µL mobile phase, mix for 5 min. Inject a 60 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherex C18 (Phenomenex USA)
Mobile phase: MeOH:THF:water 3:25:72
Flow rate: 1.0
Injection volume: 60
Detector: UV 254

CHROMATOGRAM

Retention time: 12.85
Internal standard: fludrocortisone (15.9)
Limit of detection: 5 nM

OTHER SUBSTANCES

Extracted: 11-deoxycortisol, dexamethasone, methylprednisolone, prednisolone

KEY WORDS

serum; SPE

REFERENCE
McWhinney,B.C.; Ward,G.; Hickman,P.E. Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine, *Clin.Chem.*, **1996**, *42*, 979–981.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak Plus C18 SPE cartridge with 7 mL MeOH and 14 mL water. Add 40 ng 6β-hydroxycortisone to 400 μL plasma or urine, add the mixture to the SPE cartridge, wash with 6 mL water, 3 mL MeOH:water 12:88, and 3 mL petroleum ether, elute with 5 mL ethyl acetate. Dry the eluate under reduced pressure at 40°, add 200 μL MeCN: triethylamine 90:10 and MeCN:0.1% quinuclidine 20:80 to the residue, vortex. Add 200 μL 0.02% 9-anthroyl nitrile and a few molecular sieves (4A), let stand for 30 min, evaporate under reduced pressure at 40°, dissolve the residue in 200 μL acetone, dilute with 2 mL n-hexane. Add the mixture to a Sep-Pak Plus Silica SPE cartridge, wash with 14 mL 1,2-dichloroethane, elute with 5 mL ethyl acetate. Evaporate the eluate under reduced pressure at 40°, reconstitute the residue in 200 μL mobile phase, inject a 30-60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Japan)
Mobile phase: Dioxane:ethyl acetate:chloroform:n-hexane:pyridine 58.1:11.6:11.6:16.3:2.4 (Caution! Dioxane and chloroform are carcinogens!)
Flow rate: 1 for 45 min, to 1.2 over 5 min
Injection volume: 30-60
Detector: F ex 360 em 460

CHROMATOGRAM
Retention time: 24
Internal standard: 6β-hydroxycortisone (86)
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: cortisone, 6β-hydroxycortisol, 6β-hydroxyprednisolone, prednisolone, prednisone

KEY WORDS
derivatization; plasma; urine; SPE; normal phase

REFERENCE
Shibata,N.; Hayakawa,T.; Takada,K.; Hoshino,N.; Minouchi,T.; Yamaji,A. Simultaneous determination of glucocorticoids in plasma or urine by high-performance liquid chromatography with precolumn fluorimetric derivatization by 9-anthroyl nitrile, *J.Chromatogr.B*, **1998**, *706*, 191–199.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 242.9

CHROMATOGRAM
Retention time: 17.735

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare solutions in MeCN, dilute to an appropriate concentration with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 120 × 4.6 5 μm octadecyl Bakerbond
Mobile phase: MeCN:water 30:70 containing 16 mM β-cyclodextrin
Column temperature: 5
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 0.7

OTHER SUBSTANCES
Simultaneous: testosterone, prednisone, cortisone, 17α-methyltestosterone, 17α-hydroxyprogesterone

REFERENCE
Zarzycki,P.K.; Wierzbowska,M.; Lamparczyk,H. The influence of temperature on the high performance liquid chromatographic separation of steroids using mobile phases modified with β-cyclodextrin, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1305–1311.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4.1 10 μm Versapack C18 (Alltech)
Mobile phase: MeCN:water 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: hydrocortisone, hydrocortisone acetate

REFERENCE
Michniak,B.B.; Player,M.R.; Sowell,J.W. Synthesis and *in vitro* transdermal penetration enhancing activity of lactam N-acetic acid esters, *J.Pharm.Sci.*, **1996**, *85*, 150–154.

SAMPLE
Matrix: urine
Sample preparation: Condition a 10 mL 200 mg MCF Isolute SPE cartridge with two 3 mL portions of EtOH and two 3 mL portions of water. Centrifuge urine at 4000 g for 30 in, filter

through a 0.22 μm filter unit. Dilute 0.75-3mL urine to 4 mL with water. Add 30 ng IS. Add to the SPE cartridge. Wash with three 3 mL portions of water, 3 mL MeOH:10 mM NaOH 30:70, twice with 3 mL water and with 3 mL MeOH:10 mM HCl 30:70. Elute with 3 mL EtOH. Evaporate eluate under vacuum and reconstitute the residue with 150 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.2 5 μm Nucleosil 120-C18
Mobile phase: MeCN:water 24:76
Flow rate: 0.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 10.22
Internal standard: dexamethasone (22.01)
Limit of detection: 1.7 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, cortisone

KEY WORDS
SPE; human; pig

REFERENCE
Hay,M.; Mormède,P. Improved determination of urinary cortisol and cortisone, or corticosterone and 11-dehydrocorticosterone by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *702*, 33–39.

SAMPLE
Matrix: urine
Sample preparation: Activate 3-mL 500 mg Bakerbond C18 cartridge with 2 mL MeOH and 2 mL water. Filter sample. Add 25 μL 8 μM IS in MeOH to 2 mL urine, add to the SPE cartridge, wash with two 2 mL portions of 25 mM borate buffer and with 200 mL/L acetone in water. Add 1 mL hexane and air-dry under reduced pressure for 4 min. Elute with two 1 mL portions of ethyl acetate. Dry the eluate under a stream of nitrogen and dissolve in 75 μL 400 mL/L MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher 100 C18
Mobile phase: MeCN:MeOH:water 3:43:54
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 242

CHROMATOGRAM
Retention time: 21.3-21.7
Internal standard: 6 α-methylprednisolone (39.3-40.1)
Limit of detection: 15 nM

OTHER SUBSTANCES
Simultaneous: metabolites, alprazolam, amlodipine, aspirin, carbamazepine, citalopram, corticosterone, cortisone, dexamethasone, digoxin, enalapril, ferrous sulfate, fluoxetine, furosemide, gabapentin, 5-hydroxyindoleacetic acid, lamotrigine, metyrapone, naproxen, oxazepam, oxcarbazepine, oxybutynin, phenobarbital, phenytoin, prednisone, spironolactone, valproic acid, vigabatrin, zopiclone
Noninterfering: octreotide
Interfering: prednisolone

KEY WORDS
SPE; comparison with RIA

REFERENCE
Turpeinen,U.; Markkanen,H.; Välimäki,M.; Stenman,U.-H. Determination of urinary free cortisol by HPLC, *Clin.Chem.*, **1997**, *43*, 1386–1391.

SAMPLE
Matrix: urine
Sample preparation: 10 mL Urine + 40 µL 25 µg/mL corticosterone, vortex briefly, add 1 mL 100 mM NaOH, vortex briefly, add 3 mL dichloromethane, rotate at 20 rpm for 45 min, centrifuge at 1000 g for 15 min, discard the aqueous layer, centrifuge at 1000 g for 10 min, discard the aqueous layer, add 150 mg NaCl, break up emulsion, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 150 µL MeOH, inject an aliquot.

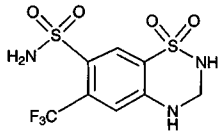
HPLC VARIABLES
Column: 150 × 3.9 4 µm Nova-Pak C18
Mobile phase: Gradient. MeOH:water from 30:70 to 44:56 over 6 min, maintain at 44:56 for 14 min, return to initial conditions over 3 min, re-equilibrate for 5 min.
Flow rate: 1
Detector: UV 246

CHROMATOGRAM
Retention time: 13.6
Internal standard: corticosterone (17.8)

OTHER SUBSTANCES
Extracted: cortisone

REFERENCE
Lee,Y.S.; Lorenzo,B.J.; Koufis,T.; Reidenberg,M.M. Grapefruit juice and its flavonoids inhibit 11β-hydroxysteroid dehydrogenase, *Clin.Pharmacol.Ther.*, **1996**, *59*, 62–71.

Hydroflumethiazide



Molecular formula: $C_8H_8F_3N_3O_4S_2$
Molecular weight: 331.30
CAS Registry No.: 135-09-1
Merck Index: 4830
Lednicer No.: 1 358

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum + 5 mL MTBE, vortex for 2 min. Centrifuge at 2700 g for 5 min and evaporate the organic phase to dryness under a stream of nitrogen. Dissolve the residue in 200 µL water, add 3 mL toluene, vortex for 2 min, centrifuge at 2700 g for 10 min, discard the toluene layer. Add 3 mL toluene, vortex, centrifuge, discard the toluene layer. Evaporate the aqueous layer to dryness under a stream of nitrogen. Reconstitute the residue in 200 µL mobile phase. Inject a 100 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm RP-C18
Column: 250 × 4 5 µm LiChrospher RP-C18
Mobile phase: MeCN:THF:200 mM pH 7.5 phosphate buffer 5:10:85
Flow rate: 1
Injection volume: 100
Detector: UV 273

CHROMATOGRAM
Internal standard: hydroflumethiazide

OTHER SUBSTANCES
Extracted: hydrochlorothiazide

KEY WORDS
hydroflumethiazide is IS; serum

REFERENCE
Vervaet,C.; Remon,J.P. Bioavailability of hydrochlorothiazide from pellets, made by extrusion/spheronisation, containing polyethylene glycol 400 as a dissolution enhancer, *Pharm.Res.*, **1997**, *14*, 1644–1646.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 25 μL 10 mg/mL hydroflumethiazide in water + 1 mL 1 M pH 10 sodium carbonate-bicarbonate buffer + 5 mL ethyl acetate, vortex 1 min, centrifuge at 1250 g for 5 min. Remove the ethyl acetate layer and evaporate at 45° under nitrogen. Dissolve in 100 μL mobile phase, inject 50 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Spherisorb ODSII
Mobile phase: MeCN:MeOH:buffer 10:9:100 (Buffer was 15.54 g tetraethylammonium hydroxide and 2.9 g 89% orthophosphoric acid in 500 mL water, pH was 2.8.)
Flow rate: 1.2
Injection volume: 50
Detector: F ex 368 em 415 or UV 271

CHROMATOGRAM
Retention time: 7.94
Internal standard: hydroflumethiazide

OTHER SUBSTANCES
Simultaneous: amiloride (detection by F), hydrochlorothiazide (detection by UV)

KEY WORDS
plasma; hydroflumethiazide is IS

REFERENCE
Van der Meer,M.J.; Brown,L.W. Simultaneous determination of amiloride and hydrochlorothiazide in plasma by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *423*, 351–357.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1mL buffer + 200 μL water + 6 mL ethyl acetate, shake for 5 min, centrifuge at 900 g for 5 min. Remove 5 mL organic layer and evaporate at 37° under a stream of nitrogen. Reconstitute with 100 μL MeOH, sonicate twice at 37° for 1 min, cool at 2-8° for 2 h to obtain a clear solution, inject a 20 μL aliquot. (Buffer was 0.38 g ammonium acetate in 500 mL water and acidified to pH 5.0 with glacial acetic acid.)

HPLC VARIABLES
Guard column: 40 × 4 35-50 μm C18 Corasil
Column: 125 × 4 5 μm Nucleosil 100-5 C18
Mobile phase: Gradient. A was MeCN:acetic acid:water 25:1:975. B was MeCN:acetic acid:water 500:1:500. A:B 100:0 to 36:64 over 16 min, re-equilibrate at 100:0 for 24 min before next injection
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 12.0
Internal standard: hydroflumethiazide

OTHER SUBSTANCES
Extracted: hydrochlorothiazide
Noninterfering: acebutolol, acenocoumarol, acetaminophen, aspirin, allopurinol, ambroxol amoxicillin, atenolol, bendroflumethiazide, benzbromarone, bezafibrate, biperiden, bisacodyl, bromazepam, butizide, caffeine, captopril, cimetidine, ciprofloxacin, clobutinol, clonidine, cotinine, diazepam, diclofenac, digitoxin, digoxin, dihydrocodeine, dihydroergotamine, diltiazem, doxepin, doxycycline, enalapril, erythromycin, fenoterol, furosemide, glibenclamide, heparin, hypoxanthine, ibuprofen, indomethacin, isosorbide mononitrate, lisinopril, lovastatin, maprotiline, methyldigoxin, methyldopa, metoclopramide, metoprolol, metronidazole, midazolam, naloxone, nifedipine, nicotine, norfloxacin, ofloxacin, oxazepam, oxipurinol, penicillin V, pentoxyfylline, phenacetin, phenazone, propyphenazone, phenprocoumon, ranitidine, salicylic acid, sotalol, sulfamethoxazole, trimethoprim, terbutaline, theophylline, tilidine, timolol, triamterene, uric acid, verapamil, ascorbic acid, warfarin, xanthine, purine and pyrimidine bases, nucleosides, nucleotides
Interfering: amiloride

KEY WORDS
plasma; hydroflumethiazide is IS

REFERENCE
de Vries,J.X.; Voss,A. Simple determination of hydrochlorothiazide in human plasma and urine by high performance liquid chromatography, *Biomed.Chromatogr.*, **1993**, *7*, 12–14.

SAMPLE
Matrix: blood, middle ear fluid
Sample preparation: 75 µL Plasma or middle ear effusion + 50 µL water, mix, add 25 µL 10% perchloric acid, vortex, add 25 µL KCl solution. Mix, centrifuge, remove supernatant, add 25 µL pH 10.4 800 mM Na_2HPO_4 to the supernatant, inject a 6 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.2 Brownlee C8 precolumn
Column: 150 × 4.6 5 µm Zorbax C8
Mobile phase: MeOH:MeCN:10 mM NaH_2PO_4 10:2:88
Column temperature: 40
Flow rate: 1.4
Injection volume: 6
Detector: UV 230

CHROMATOGRAM
Retention time: 6.4
Internal standard: hydroflumethiazide
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: amoxicillin

KEY WORDS
plasma; chinchilla; hydroflumethiazide is IS

REFERENCE
Erdmann,G.R.; Walker,K.; Giebink,G.S.; Canafax,D.M. High performance liquid chromatographic analysis of amoxicillin in microliter volumes of chinchilla middle ear effusion and plasma, *J.Liq.Chromatogr.*, **1990**, *13*, 3339–3350.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in solvent, inject an aliquot. (Solvent was 750 mg KCl in 10 mL 1 M HCl, add 400 mL water, add 400 mL MeOH, make up to 1 L with water.)

HPLC VARIABLES
Guard column: 5 × 4 7 µm Nucleosil-100 phenyl

Column: 300 × 4 7 μm Nucleosil-100 phenyl
Mobile phase: MeOH:water 40:60
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 3.1

OTHER SUBSTANCES
Simultaneous: bendroflumethiazide, degradation products

REFERENCE
Frontini,R.; Mielck,J.B. Determination and quantitation of bendroflumethiazide and its degradation products using HPLC, *J.Liq.Chromatogr.*, **1992**, *15*, 2519–2528.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 4 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:1% acetic acid 20:80
Flow rate: 1.3
Injection volume: 20 μL
Detector: UV 270

CHROMATOGRAM
Retention time: 5.280

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide
Noninterfering: captopril

REFERENCE
Papadoyannis,I.N.; Samanidou,V.F.; Georga,K.A.; Georgarakis,E. High performance liquid chromatographic determination of hydrochlorothiazide (HCT) in pharmaceutical preparations and human serum after solid phase extraction, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1671–1683.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.13

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 8.3 (A), 9.4 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine
Sample preparation: 1 mL Urine + 1 mL buffer + 200 μL water + 6 mL ethyl acetate, shake for 5 min, centrifuge at 900 g for 5 min. Remove 5 mL organic layer and evaporate at 37° under a stream of nitrogen. Reconstitute with 100 μL mobile phase, inject a 20 μL aliquot. (Buffer was 0.38 g ammonium acetate in 500 mL water and acidified to pH 5.0 with glacial acetic acid.)

HPLC VARIABLES

Guard column: 40 × 4 35-50 μm C18 Corasil
Column: 125 × 4 5 μm Nucleosil 100-5 C18
Mobile phase: MeCN:acetic acid:water 120:1:880
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 10.0
Internal standard: hydroflumethiazide

OTHER SUBSTANCES

Extracted: hydrochlorothiazide
Noninterfering: amiloride, acebutolol, acenocoumarol, acetaminophen, aspirin, allopurinol, ambroxol amoxicillin, atenolol, bendroflumethiazide, benzbromarone, bezafibrate, biperiden, bisacodyl, bromazepam, butizide, caffeine, captopril, cimetidine, ciprofloxacin, clobutinol, clonidine, cotinine, diazepam, diclofenac, digitoxin, digoxin, dihydrocodeine, dihydroergotamine,

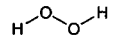
diltiazem, doxepin, doxycycline, enalapril, erythromycin, fenoterol, furosemide, glibenclamide, heparin, hypoxanthine, ibuprofen, indomethacin, isosorbide mononitrate, lisinopril, lovastatin, maprotiline, methyldigoxin, methyldopa, metoclopramide, metoprolol, metronidazole, midazolam, naloxone, nifedipine, nicotine, oxazepam, oxipurinol, penicillin V, pentoxyfylline, phenacetin, phenazone, propyphenazone, phenprocoumon, ranitidine, salicylic acid, sotalol, sulfamethoxazole, trimethoprim, terbutaline, theophylline, tilidine, timolol, triamterene, uric acid, verapamil, ascorbic acid, warfarin, xanthine, purine and pyrimidine bases, nucleosides, nucleotides

Interfering: norfloxacin and ofloxacin

KEY WORDS
hydroflumethiazide is IS

REFERENCE
de Vries,J.X.; Voss,A. Simple determination of hydrochlorothiazide in human plasma and urine by high performance liquid chromatography, *Biomed.Chromatogr.*, **1993**, *7*, 12–14.

Hydrogen peroxide



Molecular formula: H_2O_2
Molecular weight: 34.01
CAS Registry No.: 7722-84-1
Merck Index: 4839

SAMPLE
Matrix: beverages
Sample preparation: Mix 3.45 mL 500 mM pH 5.0 potassium phosphate buffer, 250 μL MeOH, and 100 μL antifoaming reagent, pass nitrogen gas through the mixture for a few min, add 1 mL beverage, add 100 μL 1000 U/mL catalase (Boehringer Mannheim) in water (purge with nitrogen before use), add 100 μL 100 mg/mL 4-amino-3-penten-2-one (Fluoral-P, Eastman) in MeCN, bubble nitrogen at 200 mL/min through the mixture, heat at 30° for 10 min, add to a Sep-Pak C18 SPE cartridge, wash with a little water, elute with 5 mL MeCN:water 50:50, inject a 20 μL aliquot of the eluate. (Prepare antifoaming reagent by diluting concentrated silicon polymer (Sigma) with water to give a 0.1% suspension.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeCN:water 50:50
Flow rate: 1
Injection volume: 20
Detector: F ex 410 em 510

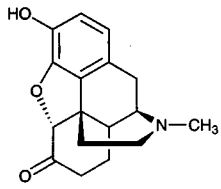
CHROMATOGRAM
Retention time: 3.5
Limit of detection: 50 ppb

OTHER SUBSTANCES
Noninterfering: ascorbic acid

KEY WORDS
derivatization; SPE

REFERENCE
Hamano,T.; Mitsuhashi,Y.; Yamamoto,S. Determination of hydrogen peroxide in beverages by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *411*, 423–429.

Hydromorphone



Molecular formula: $C_{17}H_{19}NO_3$
Molecular weight: 285.34
CAS Registry No.: 466-99-9, 71-68-1 (HCl)
Merck Index: 4847
Lednicer No.: 1 288

SAMPLE

Matrix: bile, blood, tissue
Sample preparation: 250 μL Bile, 3 mL blood, or 5 mL tissue homogenate + 1 mL 200 μg/mL nalorphine in water + 2 mL 200 mM pH 8.9 sodium borate buffer + 5 (bile) or 10 (blood, tissue) mL chloroform:isopropanol 90:10, rotate gently for 20 min, centrifuge at 2000 rpm for 10 min. Remove the organic layer and add it to 2 mL 500 mM HCl, rotate for 20 min, centrifuge for 5 min. Remove 1.8 mL of the upper aqueous phase, adjust to pH 8.6 ± 0.2 by carefully adding powdered ammonium carbonate until the solution was saturated, add 5 mL ethyl acetate: isopropanol 90:10, rotate for 20 min, centrifuge for 5 min. Remove 4.8 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm RP-18 Spheri-5
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:50 mM pH 7 phosphate buffer 40:60 Place a 70 × 2 30-38 μm Co-Pell ODS column before the injection valve.)
Column temperature: 50
Flow rate: 2
Injection volume: 20
Detector: E, Environmental Sciences Associates Model 5100, porous graphite electrode W1 900 mV W2 400 mV, difference in electrolysis current monitored

CHROMATOGRAM

Retention time: 5
Internal standard: nalorphine (14.72)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: codeine, morphine, norcodeine, normorphine
Simultaneous: acetaminophen, atropine, epinephrine, ethylmorphine, hydrocodone, hydroxyzine, naloxone, oxycodone, pentazocine, phenylpropanolamine, pseudomorphine, scopolamine, secobarbital
Noninterfering: brompheniramine, chloroprocaine, dextromethorphan, diazepam, diphenhydramine, fentanyl, flurazepam, meperidine, methadone, neostigmine, propoxyphene

REFERENCE

Hepler,B.R.; Sutheimer,C.; Sunshine,I.; Sebrosky,G.F. Combined enzyme immunoassay-LCEC method for the identification, confirmation, and quantitation of opiates in biological fluids, *J.Anal.Toxicol.*, **1984**, *8*, 78–90.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M sodium bicarbonate + 15 mL diethyl ether, rotate for 20 min, centrifuge at 800 g for 15 min. Remove the organic phase and add it to 200 μL 17 mM phosphoric acid, mix vigorously for 15 s, centrifuge for 5 min. Remove the aqueous phase and evaporate it to dryness under a stream of air at 45°, reconstitute the residue in 200 μL 17 mM phosphoric acid, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 25 × 4.6 5 μm Hi-Chrom reversible octyl (Regis)
Mobile phase: MeCN:30 mM pH 4 KH_2PO_4:0.3% sodium octanesulfonate 15:75:9

Flow rate: 1.5
Injection volume: 100
Detector: E, BAS LC4B, glassy carbon working electrode 0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8.9
Internal standard: hydromorphone

OTHER SUBSTANCES
Extracted: oxymorphone

KEY WORDS
rat; plasma; hydromorphone is IS

REFERENCE
Lam,G.; Williams,R.M.; Whitney,C.C. Electrochemical determination of oxymorphone in rat plasma by ion-pair reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 309–314.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Baxter C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 2 mL 500 mM pH 9.3 ammonium sulfate + 30 μL 1 μg/mL naltrexone, add to the SPE cartridge, wash with 3 mL 5 mM pH 9.3 ammonium sulfate, wash with 3 mL water, dry under vacuum, elute with 1 mL MeOH:triethylamine 99.5:0.5. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 100 × 3.2 5 μm Spherisorb C8
Mobile phase: MeOH:50 mM Na_2HPO_4 15:85 containing 3 mM 1-heptanesulfonic acid, pH adjusted to 3.5 with orthophosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: E, ESA Coulochem, guard cell + 650 mV, analytical cell +250 mV and +600 mV (monitored)

CHROMATOGRAM
Retention time: 8.0
Internal standard: naltrexone (16.4)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: morphine

KEY WORDS
plasma; SPE

REFERENCE
Bouquillon,A.I.; Freeman,D.; Moulin,D.E. Simultaneous solid-phase extraction and chromatographic analysis of morphine and hydromorphone in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *577*, 354–357.

SAMPLE
Matrix: formulations
Sample preparation: Add 1 tablet to 95 mL water, place on a steam bath for 15 min, cool, mix for 15 min, sonicate, allow to stand, filter, inject 13 μL aliquot

HPLC VARIABLES
Column: μBondapak C18

Mobile phase: MeOH:buffer 25:75 (Buffer was 0.01 N KH_2PO_4 + 50 mM KNO_3, adjusted to pH 4.5 with 3 N phosphoric acid.)
Flow rate: 1.1
Injection volume: 13
Detector: UV 283

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: hydrocodone, p-aminophenol, acetaminophen, codeine, p-chloroacetanilide

KEY WORDS
tablets

REFERENCE
Wallo,W.E.; D'Adamo,A. Simultaneous assay of hydrocodone bitartrate and acetaminophen in a tablet formulation, *J.Pharm.Sci.*, **1982**, *71*, 1115–1118.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1:5, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:20 mM KH_2PO_4 50:50, pH adjusted to 5.40 with 1 M NaOH
Flow rate: 1.5
Injection volume: 20
Detector: UV 216

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: morphine, ondansetron

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Trissel,L.A.; Xu,Q.; Martinez,J.F.; Fox,J.L. Compatibility and stability of ondansetron hydrochloride with morphine sulfate and with hydromorphone hydrochloride in 0.9% sodium chloride injection at 4, 22, and 32 βC, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2138–2142.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:20 mM KH_2PO_4 adjusted to pH 6.0 with 1 M KOH 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 253 ng/mL

OTHER SUBSTANCES
Simultaneous: morphine, bupivacaine

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Brownlee silica (Applied Biosystems)
Mobile phase: MeOH:10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 8.7
Limit of detection: 338 ng/mL

OTHER SUBSTANCES
Simultaneous: tetracaine, morphine

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.25
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3.72

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 mg/mL solution in 0.9% sodium chloride, dilute 1:100 with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Bakerbond C18
Mobile phase: MeOH:buffer 15:85 adjusted to pH 3.5 with o-phosphoric acid (Buffer was 15 mM sodium dihydrogen phosphate containing 3 mM 1-heptanesulfonic acid.)
Flow rate: 0.8
Detector: UV 230

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.2 5 μm Ultrasphere C18
Mobile phase: Gradient. A was MeCN containing 1 mg/mL heptanesulfonic acid. B was 50 mM pH 2.2 phosphoric acid containing 1 mg/mL heptanesulfonic acid. A:B 12.5:87.5 for 2.5 min, to 48.5:51.5 over 13.5 min, maintain at 48.5:51.5 for 4 min
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: dexamethasone, diphenhydramine, creatinine, methyl paraben, propyl paraben, degradation products

KEY WORDS
stability-indicating; buffer

REFERENCE
Walker,S.E.; DeAngelis,C.; Iazzetta,J.; Eppel,J.G. Compatibility of dexamethasone sodium phosphate with hydromorphone hydrochloride or diphenhydramine hydrochloride, *Am.J.Hosp.Pharm.*, **1991**, *48*, 2161–2166.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6

Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.84 (A), 3.42 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES
Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phos-

phoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN: buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

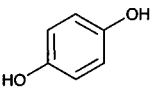
CHROMATOGRAM
Retention time: k' 7.0
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydrocodone

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

Hydroquinone



Molecular formula: $C_6H_6O_2$
Molecular weight: 110.11
CAS Registry No.: 123-31-9
Merck Index: 4853

SAMPLE
Matrix: air
Sample preparation: Condition a Sep Pak silica SPE cartridge with 10 mL dichloromethane and dry with helium at 5 L/min. Pull air through a 0.80 μm cellulose ester membrane filter and the SPE cartridge at 2 L/min for 1 h, desorb the filter with 5 mL 1% acetic acid with sonication for 10 min, elute the SPE cartridge with 5 mL 1% acetic acid, inject aliquots of the eluates.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 1% acetic acid. B was MeCN:acetic acid 99:1. A:B from 0:100 to 90:10 over 10.5 min, to 78:22 to 24.5 min, to 0:100 (step gradient), maintain at 0:100 for 5 min, re-equilibrate for 12 min.
Flow rate: 2
Injection volume: 200
Detector: F ex 304 em 338 for 6.3 min, ex 280 em 325 for 7.7 min, ex 257 em 330 for 5.3 min, ex 342 em 464 for 4.7 min, ex 285 em 310 for 11 min

CHROMATOGRAM
Retention time: 5

Limit of detection: 0.16 μg/cu.m.

OTHER SUBSTANCES
Simultaneous: catechol, cresol, 3-methylcatechol, phenol, scopoletin

KEY WORDS
SPE

REFERENCE
Risner,C.H. The quantification of hydroquinone, catechol, phenol, 3-methylcatechol, scopoletin, m+p-cresol and o-cresol in indoor air samples by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4117–4140.

SAMPLE
Matrix: formulations
Sample preparation: Emulsion. 500 μL Emulsion + 10 mL 400 μg/mL hydroquinone in MeOH + 40 mL 0.1% Tween 80, shake until homogeneous, inject a 10 μL aliquot. Drug release medium. 1 mL Drug release medium + 200 μL 100 μg/mL hydroquinone, mix, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Cosmosil 10 C18 (Nacalai Tesque)
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 2:98 for 1 min, to 45:55 over 5.5 min, maintain at 45:55 for 2 min, return to initial conditions over 1 min.
Flow rate: 2
Injection volume: 10-50
Detector: UV 220

CHROMATOGRAM
Retention time: 4.2
Internal standard: hydroquinone
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: carboplatin, epirubicin, iomeprol, mitomycin C

KEY WORDS
emulsions; drug release medium; injections; hydroquinone is IS

REFERENCE
Yamazoe,K.; Horiuchi,T.; Sugiyama,T.; Katagiri,Y. Simultaneous high-performance liquid chromatographic determination of carboplatin, epirubicin hydrochloride and mitomycin C in a Lipiodol emulsion, *J.Chromatogr.A*, **1996**, *726*, 241–245.

SAMPLE
Matrix: solutions
Sample preparation: Aqueous food simulants. Pipette 1.0 mL 200 mg/L IS in MeOH into a 25 mL volumetric flask and dilute to the mark with the food simulant obtained from migration experiment, shake. Repeat the procedure to obtain a duplicate sample, filter a portion through a 200 nm membrane filter, inject a 20 μL aliquot. Olive oil simulants. Weigh 25 g olive oil food simulant obtained from migration experiment into a beaker, pour oil into a separating funnel, allow beaker to drain for 30 s. Rinse it with 25 mL hexane and add washes to separating funnel. Add 1.0 mL 200 mg/L IS in MeOH into funnel and mix. Add 10 mL water, shake vigorously by hand for 30 s, allow to stand for 5 min. Collect aqueous phase and reextract oil with a 10 mL water. Combine aqueous extracts, make up to 25 with water, filter the extracts through a small cotton plug to remove any entrained oil. Repeat the procedure to obtain a duplicate sample. Inject a 20 μL aliquot. (Aqueous food simulants were: distilled water, 3% acetic acid in water; EtOH:water 15:85.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS

Mobile phase: MeCN:buffer 15:85 (Prepare mobile phase as follows. Dissolve 7.5 g sodium dihydrogen orthophosphate in 800 mL water, add 150 mL MeCN and adjust to pH 3.6 with glacial acetic acid. Make up to 1000 mL with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.2
Internal standard: 2-methyl-1,3-dihydroxybenzene (7.4)
Limit of detection: 300-500 ng/g

OTHER SUBSTANCES
Extracted: pyrocatechol, resorcinol

KEY WORDS
aqueous food simulants; olive oil simulants

REFERENCE
Philo,M.R.; Jickells,S.M.; Castle,L. Testing for compliance with migration limits: Determination of 1, 2-, 1,3-, and 1,4-dihydroxybenzenes in food-simulating solvents by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 746–750.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeOH:10 mM pH 5.5 potassium phosphate buffer 3.5:96.5
Flow rate: 2-3
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: catechol, phenol, phenyl glucuronide, phenyl glucoside, phenyl galactopyranoside, phenyl sulfate, resorcinol

REFERENCE
Beyer,J.; Frank,G. Hydroxylation and conjugation of phenol by the frog *Rana temporaria*, *Xenobiotica*, **1985**, *15*, 277–280.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 50 mM pH 3.0 sodium phosphate buffer
Flow rate: 1
Detector: E, ESA, Model 5020 porous graphite analytical cell, T1 0.60 V, T2 0.82 V (monitored), guard cell 0.85 V (before injector)

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: oxidized glutathione

REFERENCE
O'Gara,C.Y.; Maddipati,K.R.; Marnett,L.J. A sensitive electrochemical method for quantitative hydroperoxide determination, *Chem.Res.Toxicol.*, **1989**, *2*, 295–300.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in 1% acetic acid.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere-ODS C18
Mobile phase: Gradient. A was MeCN:acetic acid 99:1. B was 1% acetic acid in water. A:B from 0:100 to 10:90 over 10 min, to 20:80 over 25 min, wash with A for 6 min, re-equilibrate for 14 min.
Flow rate: 2
Injection volume: 20
Detector: F ex 304 em 338

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: catechol (F ex 280 em 325), phenol (ex 274 em 298), resorcinol (F ex 284 em 313)

REFERENCE
Risner,C.H.; Cash,S.L. A high-performance liquid chromatographic determination of major phenolic compounds in tobacco smoke, *J.Chromatogr.Sci.*, **1990**, *28*, 239–244.

SAMPLE
Matrix: urine
Sample preparation: Condition a 500 mg Bond Elut SAX SPE cartridge with 3 mL MeOH and 3 mL water. Dilute 125 μL urine to 4 mL with water, adjust to pH 4.5 with ascorbic acid, add 12.5 μL enzyme solution, heat at 37° for 48 h, add to the SPE cartridge, wash with 3 mL 5 mM pH 7 phosphate buffer. Acidify the eluate to pH <3 with concentrated HCl, add 5 mL ether, vortex, repeat the extraction twice. Combine the organic layers and evaporate them to dryness under reduced pressure at 30°, reconstitute the residue in 1 mL 1% aqueous phosphoric acid, inject a 20 μL aliquot. (The enzyme solutions used to deconjugate glucuronides and sulfate esters were β-glucuronidase/arylsulfatase (Merck, 4114), arylsulfatase (Sigma, S 1629), and β-glucuronidase diluted 1:6 with water (Boehringer, 127051).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeOH:5 mM pH 3.4 phosphate buffer 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 3.2
Limit of detection: 60 μg/mL

OTHER SUBSTANCES
Extracted: catechol, phenol

KEY WORDS
mouse; SPE

REFERENCE
Schad,H.; Schäfer,F.; Weber,L.; Seidel,H.J. Determination of benzene metabolites in urine of mice by solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *593*, 147–151.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.2 μm), inject an aliquot directly. Hydrolyze conjugates by heating with 6 M HCl at 37° for 18 h, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:1.5% trifluoroacetic acid on water 10:90
Flow rate: 0.5
Detector: UV or radioactivity

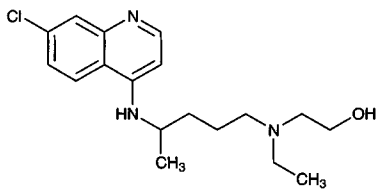
CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Extracted: phenol, phenyl glucuronide, phenyl sulfate

KEY WORDS
rat

REFERENCE
Hughes,M.F.; Hall,L.L. Disposition of phenol in rat after oral, dermal, intravenous, and intratracheal administration, *Xenobiotica*, **1995**, *25*, 873–883.

Hydroxychloroquine



Molecular formula: $C_{18}H_{26}ClN_3O$
Molecular weight: 335.88
CAS Registry No.: 118-42-3, 747-36-4 (sulfate)
Merck Index: 4863
Lednicer No.: 1 342

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 5 M NaOH + 100 μL 10 μg/mL chloroquine in MeOH + 5 mL hexane:diethyl ether 50:50, vortex for 1 min, centrifuge at 1000 g for 10 min, freeze in dry ice/acetone, remove the organic layer. Thaw out the aqueous layer and repeat the extraction. Combine the organic layers and evaporate them to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm cyano (Regis)
Column: 75 × 4.6 3 μm Ultremex cyano (Phenomenex)
Mobile phase: 20 mM Dimethyloctylamine phosphate:60 mM ammonium acetate 40:60, pH adjusted to 4.5 (Dimethyloctylamine phosphate was prepared by adding phosphoric acid to N,N-dimethyloctylamine to precipitate the salt.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 320

CHROMATOGRAM
Retention time: 15
Internal standard: chloroquine (23)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites